Current Drug Targets - Inflammation & Allergy, 2002, *1*, 377-392

TNFα as Therapeutic Target: New Drugs, More Applications

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Abstract: TNFα is a crucial cytokine in the establishment and maintenance of inflammation in multiple autoimmune diseases. With the introduction of infliximab and etanercept, two injectable biologic TNFα blocking drugs are now available. Both are effective in the treatment of rheumatoid arthritis, reducing clinical inflammation and damage to bones. In addition, infliximab is FDA-approved for the treatment of Crohn's disease. More recent controlled trials have shown effectiveness for TNFα blockers in psoriasis, psoriatic arthritis, and ankylosing spondylitis. Further trials are underway in diverse inflammatory conditions including including uveitis, sarcoidosis, Behcet's syndrome, and graft versus host disease. Although the safety profile has been generally excellent, the rare development of reactivation tuberculosis, anti double-stranded DNA antibodies, or a demyelination syndrome point out the need for further close follow-up of treated patients. New formulations of recombinant anti-TNFα biologics undergoing clinical trials use modifications to reduce antigenicity, increase the half-life, and maintain or extend the efficacy of these agents. Future development of TNFα antagonists is turning to small molecule inhibitors. The inhibition of the TNFα signaling cascade is under study using blockers of the p38, JNK, and ERK kinases, and by antagonists of transcription factor NF-κB activation. The goal of this approach is to develop compounds that are orally available, have increased selectivity compared to generalized blockade of TNFα, yet are therapeutically useful for a range of chronic inflammatory diseases.

Keywords: TNFα, Biologics, Cytokines, Rheumatoid Arthritis, p38 kinase.

INTRODUCTION

The recognition of TNFα as a target of therapy in inflammatory diseases stems from three decades of research on the immune system pathways set in motion by this cytokine. TNFα is produced mainly by cells of the monocyte/macrophage lineage, although T cells (Th1), neutrophils, mast cells, eosinophils, and activated endothelium can also contribute to its production. The stimuli that elicit TNFα are legion but are generally inflammatory or noxious signals: contact with activated T lymphocytes, cytokines, UV light, heat, X-irradiation, and chemicals. Rapid release of TNFα results from cleavage of pre-formed, membrane-bound cytokine through the action of TACE (TNFα converting enzyme) and through the extrusion of mast cell and eosinophil granules containing TNFα. New synthesis of TNFα accounts for the later release of further TNFα, after transcription factors such as NF-κB, c-Jun, ATF-2, Ets, and NFAT are activated on the TNFα promoter, in part by the signal transduction cascades set in motion by TNFα itself [1] (Fig. (1)).

The focus on TNFα in the treatment of inflammatory conditions stems from a dissection of the roles of multiple inflammatory cytokines. In studies of rheumatoid synovium, the cytokines include TNFα, IL-1, IL-6, and GM-CSF, with similar inflammatory effects described for several of these. The redundancy of cytokine actions at first made it unclear if any single cytokine had a unique role in an inflammatory response. In addition, the proinflammatory actions of some cytokines and chemokines were partially inhibited by the anti-inflammatory effects of other cytokines such as IL-4, IL-10, and TGF-β, as well as the release of soluble receptors (for TNFα, IL-and IL-2) as well as the IL-1 receptor antagonist. Clearly, these downregulatory mechanisms were overwhelmed in states of inflammation, but presented targets for upregulation by new treatment strategies.

The use of anti-cytokine antibodies allowed a dissection of roles for one or several cytokines at a time. In separate studies of sepsis and of rheumatoid synovium, treatment with antibodies to TNFα was found to reduce the production of other inflammatory cytokines such as IL-1 and IL-6 [2, 3]. Therefore, despite the apparent complexity of the cytokine network, targeting of key factors could dampen the entire inflammatory response. Beginning with this observation, TNFα has been targeted by recombinant antibodies that neutralize it and by soluble receptors that remove it from the circulation. Based on *in vitro* studies and animal trials, the initial disease targets for TNFα blockade were rheumatoid arthritis and Crohn's disease. In subsequent years, the list of inflammatory diseases in which these drugs are being studied has expanded tremendously. In addition, recombinant anti-TNFα proteins are being modified to

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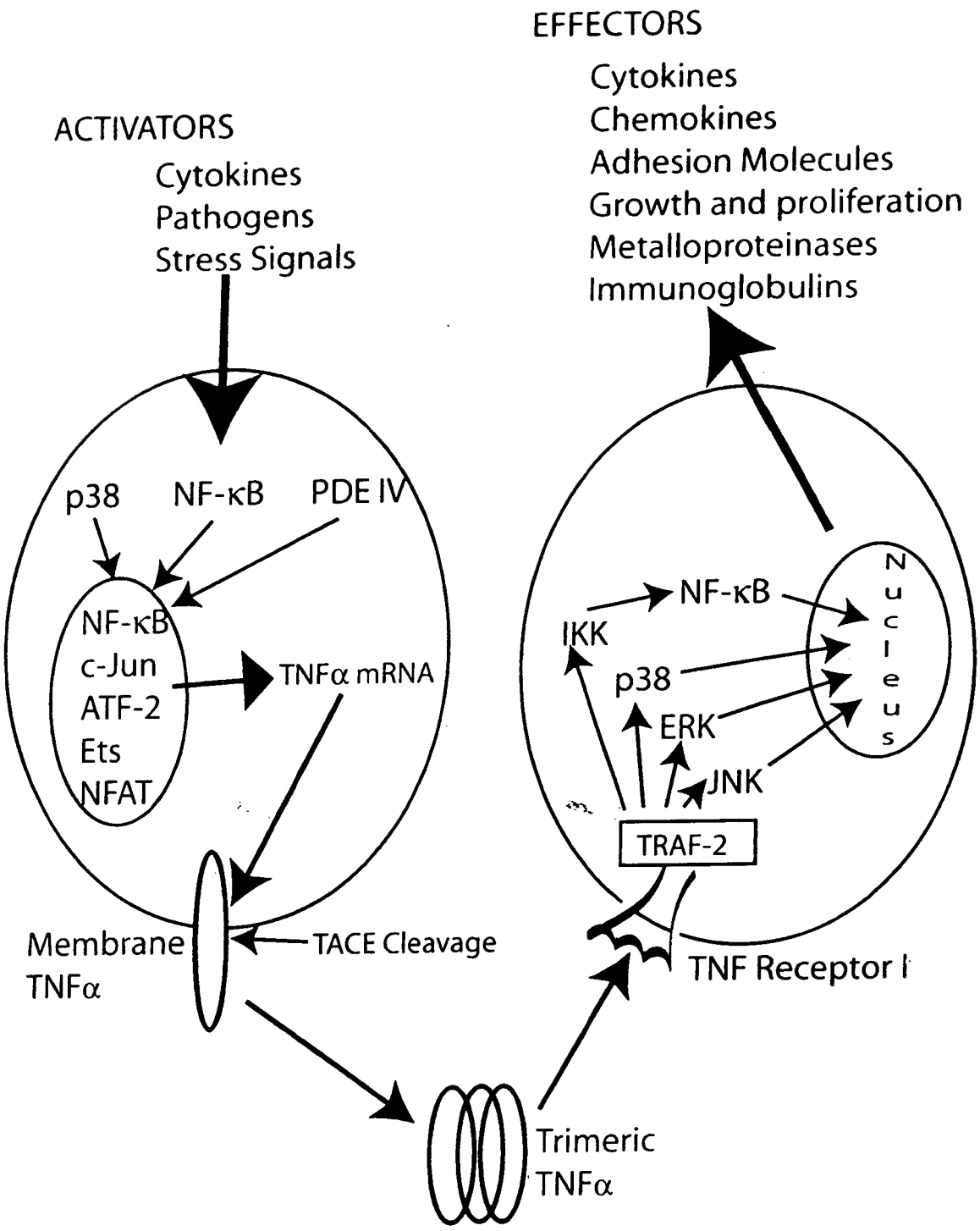

Fig. (1). TNFα Pathway. Activators such as cytokines, infectious pathogens, or stress signals initiate kinase pathways that lead to the activation of p38, NF-κB, and phosphodiesterase IV (PDE IV), among others. These in turn activate transcription factors in the nucleus known to be important in TNFα gene expression (NF-κB, c-Jun, ATF-2, Ets, NFAT). The TNFα molecules can be expressed as a transmembrane form, or this form can be cleaved to produce circulating TNFα. When TNFα trimers engage the TNFα Receptor I on effector cells, a signaling cascade is activated that includes TRAF-2 and the more downstream kinases IKK, p38, ERK, and JNK. These signals are transmitted to transcription factor in the nucleus, where numerous inflammatory genes are activated. The resulting elaboration of cytokines and chemokines regulates adhesion molecule expression, cellular growth, proliferation and differentiation, metalloproteinase production, and immunoglobulin generation.

obtain easier dosing, longer half-lives, and potentially improved efficacy, leading to new generations of biologic TNFα blockers. With the focus on TNFα as a key mediator of inflammatory signals, there has also been an intensive effort to identify targets within the TNFα signal transduction cascade for inhibition by small molecules. In this review, the focus will be on anti-TNFα molecules that are FDA-approved, undergoing human trials, or nearing human trials after preliminary animal studies in the treatment of inflammatory diseases.

ANTI-TNFα BIOLOGICS

Only ten years have elapsed since the first trials of anti-TNFα therapy were initiated in humans. In subsequent years, double-blind, placebo-controlled, randomized trials have demonstrated the clinical efficacy of the two available agents, etanercept and infliximab. Etanercept is a fully human recombinant fusion protein of the p75 TNF receptor II with a human IgG1 Fc tail. On the other hand, infliximab is a chimeric monoclonal antibody to TNFα in which the murine constant region has been replaced by human sequences . Both agents have won FDA approval for the treatment of rheumatoid arthritis, and infliximab is indicated for treatment of Crohn's disease. Recently, treatment of psoriatic arthritis has been approved for etanercept, and further applications are under active study.

USE IN RHEUMATOID ARTHRITIS (RA)

Infliximab

The major clinical study using infliximab has been the ATTRACT trial, with data out to 102 weeks of follow-up [4, 5]. The design was that of a randomized, double-blind, placebo-controlled, international multicenter study in 428 patients. The patients were median age 54, 78% female, had a disease duration of median 8.4 years, and half were in functional class III or IV. The patients had long-standing RA that had not responded fully to previous therapies, with a median use of 3 prior DMARDs, and with 51% of patients on methotrexate for at least 3 years. In the study, a stable methotrexate dose (median 15 mg/week) was given to all patients, and the five study groups received placebo infusions, 3 mg/kg infliximab infusions every four or eight weeks, or 10 mg/kg infliximab infusions every four or eight weeks. The primary endpoints were to assess improvement in signs and symptoms of RA, to determine structural damage on X ray, and to quantify the improvement in physical function and disability.

The results of the ATTRACT trial were evaluated at weeks 30, 54, and 102. Clinical improvement of the RA disease activity was quantitated using the ACR response criteria. Patients are considered to have an ACR 20 response if they have a 20% improvement in the counts of tender joints and of swollen joints, plus 20% improvement in 3 of 5 further assessment categories (patient pain assessment, patient global assessment, physician global assessment, Health Assessment Questionnaire, ESR or CRP value). ACR 50 and ACR 70 responses similarly indicate a 50% or 70% improvement in these categories, respectively. The results demonstrated that by 30 weeks, there was a 50-58% improvement in ACR 20 for the four infliximab groups, versus a 20 % improvement for the placebo group on methotrexate alone. There was no statistical difference between the different infliximab doses and dosing intervals, resulting in FDA approval for initial infliximab dosing at 3 mg/kg every 8 weeks after 3 loading doses. The results have remained consistent through 54 weeks and 102 weeks of assessment, with ACR 20 responses of 40-48% in the infliximab groups and 16% in the placebo group at the last assessment. Overall, patients treated with infliximab infusions derive three benefits from the concomitant use of methotrexate: improved rates of response, longer duration of response, and less development of antibodies to infliximab (HACA, human anti-chimeric antibodies). Methotrexate clearly has immunosuppressive activity in minimizing HACA formation. By making it less likely that infliximab will lose its therapeutic efficacy due to neutralization by HACAs, the rate and duration of clinical responses are improved in the presence of methotrexate. It has not been tested whether methotrexate contributes any therapeutic benefits not attributable to the prevention of HACAs at the low doses used in combination with infliximab.

Radiographic progression of disease was evaluated in the ATTRACT trial by using a modified Sharp scoring system. Two readers scored each patient's X rays in a blinded fashion, recording joint erosions in 44 joints and joint space narrowing in 40 joints. All patients had baseline joint damage, consistent with their long-standing rheumatoid arthritis. An arrest of radiologic progression was evident by 30 weeks in all four infliximab groups and was maintained through 54 and 102 weeks. The benefit was seen as reduced bone erosion as well as reduced cartilage damage. Interestingly, radiographic change was arrested even in the subgroup of infliximab-treated patients with little clinical improvement by ACR 20 criteria. On the other hand, the methotrexate plus placebo group had steady deterioration of their X ray findings. At 30, 54, and 102 weeks, these patients had worsening of the Sharp score by 4.8, 7.0, and 12.6 units, respectively.

Assessment of physical and functional disability in the ATTRACT trial was by use of HAQ (Health Assessment Questionnaire) and the SF-36. The HAQ is an 8-component measure of daily function, including scoring of dressing and grooming, arising, eating, walking, hygiene, reach, grip, and activities. The SF-36 is a quality of life measure that includes a mental component and a physical component summary score. By week 102 in the ATTRACT trial, the HAQ improvement for the methotrexate plus placebo group was 0.1 units, versus 0.3 to 0.4 units for the four infliximab groups, which was statistically significant. A clinically meaningful improvement is > 0.25 points, correlating with lower health care costs and a lower likelihood of losing one's job. For the SF-36 at 102 weeks, the mental component achieved borderline statistically significant improvement in two of the four infliximab groups and the physical component was significantly improved in all four infliximab groups.

Adverse events in the ATTRACT trial were seen at similar rates in the infliximab and placebo groups. Mild

infusion reactions were seen in 5% of patients and only rarely included hypotension or pre-syncopal reactions. There was no statistical difference in infections, including upper respiratory infection, pneumonia, and sepsis. On the other hand, post-marketing surveillance has demonstrated an increased incidence of tuberculosis in infliximab recipients, felt because of its occurrence within 14 weeks of infliximab initiation to be reactivation of previously latent disease [6]. Because of this association, skin testing for tuberculosis is now mandatory in all patients beginning infliximab therapy, with treatment for all positive results. The occurrence of reactivation TB in infliximab recipients appears specific to this TNF-α blocker, since a similar association has not been found using etanercept. Therefore, there is speculation that the mechanism for this effect stems from infliximab's tight binding to target cells and a potential for lysing macrophages that might harbor latent TB.

Etanercept

Etanercept represents a second approach to targeting TNFα for neutralization. The etanercept molecule consists of two recombinant copies of the human soluble p75 TNF receptor fused to the Fc portion of human IgG. Etanercept's half-life is roughly half as long as that of infliximab (4.8 days vs. 9.8 days), it binds to both TNFα and lymphotoxin-α (infliximab binds only TNFα), and it does not fix complement or lyse cells, unlike infliximab. Despite such subtle differences, clinically the two drugs have produced similar results in rheumatoid arthritis.

Etanercept has been compared to placebo therapy in a trial of 234 patients who had rheumatoid arthritis for over 10 years on average and had failed multiple medications [7]. In the subgroup treated with etanercept 25 mg s.c. b.i.w., the ACR 20 response at 6 months was 59% versus 11% for placebo, while the ACR 50 response was 40% versus 5%, respectively. The patient population and the resulting clinical responses were comparable for this trial of etanercept and the ATTRACT trial studying infliximab.

Etanercept was also studied in early rheumatoid arthritis in a 2 year study with an open-label third year extension (ERA-Early Rheumatoid Arthritis) [8]. In this blinded study of previously untreated RA, 632 patients were enrolled to receive etanercept at 10 mg s.c. b.i.w. or 25 mg s.c. b.i.w., or standard treatment of methotrexate escalated to 20 mg p.o. weekly. The ACR 20 response was comparable for methotrexate (64%) and etanercept treatment (69% for the two groups) in the first year and was maintained through the second year [9]. Therefore, both methotrexate and etanercept were highly effective medications for active, early, previously untreated RA.

The ERA trial was the first large etanercept trial to include evaluation of radiographs. Using a modified Sharp scoring system for the X rays (bone erosions scored in 46 joints and joint space narrowing in 42 joints), the joint space narrowing score was similar in the methotrexate and 25 mg etanercept groups at one year, while the joint erosion score deteriorated significantly less in the 25 mg etanercept group (0.47 units vs. 1.03 units, p = 0.002). Overall, the deterioration in the 25 mg etanercept group has been calculated as 1.3 Sharp units/year, compared with 3.2 Sharp units/year for the methotrexate group, and an expected deterioration of 16-18 Sharp units in untreated patients [10]. In year 3, when the methotrexate patients were switched to etanercept 25 mg b.i.w., deterioration of Sharp scores was nearly halted [11]. Side effects from etanercept treatment were predominantly injections site reactions. The conclusion from this trial is that methotrexate monotherapy and etanercept are both highly effective therapies in early RA. Etanercept is more effective in the rate of improvement in clinical symptoms and is more likely to result in ACR 20, 50, and 70 responses in disease activity. For joint damage, etanercept is again statistically superior to methotrexate in halting the progression of joint erosions.

Substantial limitations remain in the treatment of rheumatoid arthritis by TNFα blockers. The etiology of the underlying disease remains unknown and treatment controls the inflammatory manifestations without achieving a true cure. When treatment is stopped, the arthritis flares again in most cases. The relentless bony damage and destruction is arrested by these treatments in most patients, yet the clinical response even as measured by the relatively lax criterion of an ACR 20 response is achieved by about 60% of patients, leaving 40% as treatment failures. By the use of other drugs and combinations of treatments, further patients respond but there is a need for identifying further drug targets and therapeutic modalities.

TNFα BLOCKERS AND CROHN'S DISEASE

TNFα represents a primary pathogenic cytokine in the intestinal inflammation of Crohn's disease. TNFα is found at high levels in the gut mucosa, leading to production by gut fibroblasts of both matrix degrading enzymes and proliferative stimuli (from epithelial growth factors such as keratinocyte growth factor) causing crypt cell hyperplasia. Mouse models suggest that T cell responses in the gut are misdirected at gut flora, causing inflammatory disease (reviewed in [12]). Blockade of TNFα by i.v. infusion of infliximab reduces the intestinal levels of TNFα but also resets the Th1/Th2 cytokine balance. Patients that have responded to treatment have decreased Th1 responses, including elaboration of IFN-γ and TNFα. The fact that favorable responses can last up to one year after anti-TNFα infusions indicates that the therapeutic effects are not solely due to one-time removal of TNFα but rather involve a broader immunomodulatory effect. Treatment alters the cytokine milieu in the intestine to allow a more normal Th1/Th2 balance and therefore resolves the excess production of Th1 cytokines TNFα, IFN-γ, and IL-12 [13].

Infliximab was approved by the FDA in 1998 for treatment of Crohn's disease. Two multicenter, randomized, double-blind, placebo-controlled trials have been conducted to demonstrate the effectiveness of infliximab. In a 12 week, double-blind, placebo-controlled trial, infliximab was given to 108 patients with moderate to severe Crohn's, using single doses of 5 mg/kg, 10 mg/kg, 20 mg/kg, or placebo [14]. There was no dose response, but there was a highly significant clinical response in the Crohn's Disease Activity

Index (CDAI) by week 4 in the combined infliximab groups (65% improved > 70 points on CDAI) versus the placebo group (17% response; $p < 0.001$). The best response was seen for the 5 mg/kg dose, where 81% of recipients met clinical response criteria. The improvement was maintained through week 12 (41% infliximab vs. 12% placebo, $p = 0.008$). A remission was achieved at week 4 by 33% of infliximab and 4% of placebo patients ($p = 0.005$), while the same trend but not the statistical significance was retained at week 12 (24% remissions on infliximab vs. 8% on placebo, $p = 0.31$).

The effectiveness of infliximab in treating fistulas in Crohn's disease was assessed in a further randomized, double-blind, placebo-controlled trial [15]. Here, 94 patients with Crohn's disease and fistulas for at least 3 months received placebo or infliximab in 3 doses of 5 or 10 mg/kg at weeks 0, 2, and 6. After 18 weeks of follow-up, there was complete closure of fistulas in 46% of infliximab-treated patients and in 13% of placebo patients ($p=0.01$). No dose response was seen, and the median duration of the response was 12 weeks. Since the response wanes without further dosing, a large placebo-controlled trial was recently presented to address the utility of infliximab maintenance therapy in Crohn's disease [16]. Patients who responded to an initial infliximab dose within 2 weeks were randomized to receive placebo or infliximab (5mg/kg or 10 mg/kg) infusions at weeks 2 and 6, and then every 8 weeks. The infliximab groups showed two benefits: a greater likelihood of remaining in remission at weeks 30 and 54, and a higher rate of steroid discontinuation ($p \leq 0.007$ for both endpoints). The two infliximab doses were equally effective. Therefore, infliximab maintenance therapy has proven effective in Crohn's disease.

Etanercept also has potential benefit in the treatment of Crohn's disease, based on early uncontrolled studies (e.g. [17]). However, in a recent placebo-controlled trial of etanercept in Crohn's disease, the drug was found safe but not effective [18]. Using the dose of 25 mg s.c. b.i.w. established for the treatment of rheumatoid arthritis, 43 patients with moderate to severe Crohn's were assessed in an 8-week trial. The primary outcome assessment at week 4 showed no difference in clinical response between the placebo group (45%) and the etanercept group (39%, $p=0.763$). It remains unknown whether higher dosing or more frequent administration would provide clinical benefit in this patient group.

A humanized antibody to TNFα is also under study in the treatment of Crohn's disease. CDP571 is a human IgG4 molecule whose CDR has been replaced by that of a murine anti-human TNFα monoclonal antibody. Unlike infliximab, the IgG4 moiety does not allow fixation of complement or antibody-dependent cell cytotoxicity [19]. In a randomized controlled trial of CDP571 in Crohn's disease, the clinical effect on CDAI scores was evaluated 2 weeks after infusion of a single dose of CDP571 at 5 mg/kg. A significant improvement in the CDAI was seen in the CDP571 group (reduction of 96 points) versus that seen in the placebo group (reduction of 6 points, $p=0.0003$) [20]. The beneficial effect was lost by week 8 after the infusion. In a larger, 24 week, randomized, double-blind, placebo-controlled trial, 169 patients with Crohn's were treated with placebo or CDP571 at 10 or 20 mg/kg [21]. The primary endpoint was a reduction in the CDAI score of at least 70 points by week 2, and this was achieved by 45% of CDP571 patients and 27% of placebo patients ($p=0.023$). In addition, patients were then retreated every 8 or 12 weeks over 24 weeks. In this group of Crohn's patients that had failed other therapies, the CDP571 treated patients showed a trend towards greater median time to withdrawal from the study and greater rates of clinical remission, although these did not reach statistical significance. A further randomized controlled trial is underway to investigate the steroid-sparing effects and the maintenance of improvement in CDP571-treated patients. Studies are also needed in assessing the use of TNFα blockade in maintaining remissions and minimizing the use of other medications such as steroids.

TNFα BLOCKADE IN SPONDYLOARTHROPATHIES

Ankylosing Spondylitis

Although less extensive than studies of rheumatoid tissue, *in vitro* studies and animal models also suggested the relevance of TNFα in the pathogenesis of ankylosing spondylitis (AS). Messenger RNA for TNFα is overexpressed in the inflamed sacroiliac joints in patients with adult AS or juvenile spondyloarthropathy, at levels that are as high or higher than those found in control patients with RA [22-24]. The source of cytokine mRNA in AS joints is felt to be the T-cells and macrophages present in sacroiliac lesions, a pathogenesis similar to inflamed RA joints [25]. The circulation of AS patients contains elevated levels of the proinflammatory cytokines TNFα and IL-6, a pattern that is similar to the inflammation found in RA [26]. In a mouse model, transgenic overexpression of TNFα resulted in axial spinal disease and an enthesopathy resembling human AS [27]. Finally, inflammatory bowel disease is itself associated with a spondyloarthropathy involving the axial and peripheral skeleton. The intestinal mucosa in Crohn's disease expresses large amounts of TNFα, for which TNFα blockade provides effective treatment [28]. This evidence provided parallels between the arthritis seen in spondyloarthropathies and that associated with inflammatory bowel disease.

The treatment of AS has been unsatisfactory, with poorer patient response than in the treatment of RA. Standard therapies include physical therapy to maintain mobility and NSAIDs for relief of pain and inflammation, although such interventions are not felt to eliminate disease progression. Sulfasalazine has some limited benefit, especially in early and active disease, and for the treatment of peripheral arthritis and the prevention of anterior uveitis [29]. Methotrexate has also been used to improve pain and stiffness, but controlled studies have not been performed. Many patients have progressive disease or insufficient relief of pain and stiffness despite these measures. With this background, trials of TNFα blockers were begun in the spondyloarthropathies.

Short-term trials have studied infliximab infusions for the treatment of AS. Infliximab was given at 5 mg/kg i.v. at

time 0, 2 weeks, and 6 weeks. Uncontrolled studies of 11 patients showed rapid improvement in spinal pain, fatigue, and morning stiffness, improvement in quality of life measures (by SF-36) at 4 weeks, and improvement in physical functioning [30]. With no further infusions of infliximab planned in this study, symptoms returned after a mean of 6 weeks. Next, a double-blind, placebo-controlled, multicenter study was reported using the same regimen of three infliximab infusions and a 12 week observation period [31, 32]. The primary endpoint in this 70 patient study was a 50% improvement in the BASDAI (Bath AS Disease Activity Index), a measure of fatigue, spinal pain, joint pain and swelling, localized tenderness, and morning stiffness duration. The results showed that 53% of the infliximab patients and 9% of placebo patients achieved 50% improvement in the BASDAI. In addition, other study measures including the BASFI (Bath AS Functional Index), a metrology index, the health-related quality of life measurement, and the CRP (C-reactive protein) were also significantly improved in the infliximab group. A second double-blind, placebo-controlled randomized trial studied 40 spondyloarthropathy patients given infliximab 5 mg/kg at weeks 0, 2 and 6 versus placebo. In the abstract describing the results at 12 weeks, the investigators found infliximab statistically significantly superior to placebo in patient global assessment, physician global assessment, laboratory measures, and spinal and peripheral arthritis [33]. Recognizing that AS patients have recurrence of symptoms in the weeks after the last infliximab infusion, an open trial has been reported in which a maintenance infusion of 5 mg/kg infliximab was given every 14 weeks after the initial 3 doses, with maintenance of clinical benefit although clinical symptoms recurred before the retreatment infusions in a growing percentage of patients (16% at week 20, 79% at week 48) [34]. As has also been seen in the treatment of RA patients, both an increase in the dose and a decrease in the interval between infusions may be useful in the maintenance of a clinical response.

Etanercept has also been studied in the treatment of AS. One randomized, double-blind, placebo-controlled trial has been presented [35]. In a four month study, 40 patients with active disease and morning stiffness over 45 minutes were randomized to receive placebo or twice weekly etanercept (the standard dose for treatment of RA). The primary outcome measure was a greater than 20% improvement in more than 3 of these indicators: morning stiffness, nocturnal spinal pain, BASFI, patient global assessment, or swollen joint count. The results showed 80% of etanercept patients achieved this 20% response, while 30% of placebo patients did ($p < 0.004$). In addition, there were significant improvements in morning stiffness, nocturnal spine pain, BASFI, and patient global assessment in the etanercept group. An uncontrolled trial in 10 patients has added a semiquantitative MRI scoring system to the evaluation of AS patients [36]. In this 6 month study using etanercept 25 mg s.c. b.i.w., the MRI of the sacro-iliac joints, spine, and peripheral joints was evaluated at baseline and at week 24. Correlating with clinical improvement, the MRI demonstrated diminished bone edema in SI joints, resolution of some entheseal lesions, and decreased spinal edema. These changes in bone edema and reduction in entheseal lesions may be the basis for relief of pain and stiffness in AS patients with long-standing disease in whom plain X-ray can demonstrate no response to therapy. In the future, if objective diminution in the bony lesions of AS can be demonstrated in controlled studies, TNFα blockade may have a role in ameliorating symptoms of long-term AS patients and possibly also in preventing the progression of structural damage in more recently diagnosed patients.

Psoriatic Arthritis

Psoriatic arthritis can have similarities to RA in its involvement of hand joints, occurrence as a symmetric polyarthritis, and potential for severe joint destruction. However, it is classified as a spondyloarthropathy because of features such as spondylitis (not seen in RA), occurrence as an oligoarthritis, and seronegative status (lack of rheumatoid factor). TNFα has been implicated in the pathogenesis of psoriatic arthritis on the basis of similarities in clinical presentation to RA as well as the demonstration of TNFα and TNFα receptor in psoriatic arthritis synovial fluid, synovium, and psoriatic skin lesions [37-39]. Success in the treatment of RA with TNFα blockers has provided further impetus to applying the same therapy in psoriatic inflammatory disease.

The skin disease of psoriasis has been evaluated in a double-blind, randomized trial of 33 patients receiving infliximab for plaque type psoriasis [40, 41]. Two dose levels of infliximab, 5 mg/kg or 10 mg/kg, were infused at weeks 0, 2, and 6. Patients were evaluated at week 10 for physician global assessment, National Psoriasis Foundation Psoriasis Score (NPF-PS), the Psoriasis Area and Severity Index (PASI), and biopsies of psoriatic skin lesions. The responders were those with good, excellent, or clear ratings on the physician global assessment. The placebo group had 2 of 11 responders, low dose infliximab had 9 of 11 responding, and higher dose infliximab had 10 of 11 responding ($p < 0.01$). Skin biopsies showed decreased skin thickness and epidermal CD3+ cell infiltrates in the infliximab but not the control groups.

Infliximab has not been studied in the treatment of psoriatic arthritis in controlled trials, but open-label studies indicate benefit through one year of follow-up [42]. On the other hand, etanercept has undergone controlled trials and was approved by the FDA for treatment of psoriatic arthritis. In a phase II trial, 60 patients with psoriatic arthritis and skin disease received the standard dose of etanercept (25 mg s.c. b.i.w.) or placebo injections for 3 months, with a 6 month open-label extension [43]. The primary endpoint was the PsARC (Psoriatic Arthritis Response Criteria), with secondary criteria of ACR 20, 50, or 70 responses, Health Assessment Questionnaire, target lesion response, and Psoriatic Area and Severity Index. The results showed that the etanercept group had a 87% PsARC response versus 23% for placebo ($p < 0.001$). Significant improvement was also noted in the ACR responses, tender and swollen joint counts, HAQ, and PASI. Sustained improvement was noted in the 6 month open-label follow-up, with 25% of participants discontinuing methotrexate and 44% stopping steroid use. A Phase III, randomized, double-blind, placebo-controlled, multicenter, 6-month trial of etanercept has been

completed with 205 psoriatic arthritis patients [44]. In this study, the primary endpoint was the ACR 20 response at 3 months, with secondary endpoints at 6 months of ACR 20, 50, and 70, PsARC, HAQ, SF-36, PASI, and assessments of skin lesions. The primary endpoint showed a 59% etanercept response in ACR 20 at 3 months, versus 15% for placebo ($p < 0.001$). The response was maintained at 6 months with an ACR 20 of 50% (vs. 13% for placebo), an ACR 50 of 37% (vs. 4% placebo), and an ACR 70 of 9% (vs. 1% placebo). Among other secondary endpoints, the PsARC, tender and swollen joint counts, HAQ, target lesion score improvement, and PASI score improvement were all significantly improved in the etanercept group. Therefore, etanercept is effective for the treatment of both the skin disease and the arthritis in psoriasis.

NEW GENERATIONS OF BIOLOGIC TNFα BLOCKERS

Given the clinical efficacy of infliximab and etanercept, there has been interest in developing further antibody compounds to treat rheumatoid arthritis. The potential benefits compared to the available agent would be a prolonged half life to allow subcutaneous administration by the patient less frequently than twice a week, lower immunogenicity, at least equivalent clinical efficacy, and possibly lower cost (etanercept currently costs $12,000/year; infliximab costs $9,000/year plus infusion charges). Competition among pharmaceutical companies may also drive new product development in this area. The biological products under development follow the basic categories that classify infliximab and etanercept: monoclonal anti-TNFα antibodies (D2E7/adalimumab, CDP571, CDP870) and soluble TNFα receptors (PEGylated sTNF RI dimers and monomers, lenercept), respectively (Table I).

Table I. Biologicals that Block TNFα

Agent	Etiology
Monoclonal anti-TNFα Antibodies	
Infliximab	Mouse/human chimera
CDP571	Humanized, murine CDR3
Adalimumab (D2E7)	Fully human
CDP870	PEGylated Humanized Fab fragment
Soluble TNFα Receptors	
Etanercept	TNFα p75 Receptor-Fc fusion
Lenercept	TNFα p55 Receptor-Fc fusion
PEGylated p55 Receptor	Receptor dimers
PEGylated p55 Truncated Receptor	Receptor monomers

Among the anti-TNFα antibodies, adalimumab or D2E7 is a fully-human recombinant monoclonal antibody. The antibody is of the IgG1 isotype and has an estimated half-life of 6 to 13.7 days. In a three month, phase II dose-finding study of 238 RA patients, adalimumab was given at 20 mg, 40 mg, or 80 mg sc weekly and compared to placebo injections [45]. There was a statistically significant benefit from adalimumab treatment, with ACR 20 responses of 49%, 57%, and 56%, respectively, in the three adalimumab groups, versus 10% in the placebo group. When given over one year at 0.5 to 10 mg/kg s.c. biweekly, adalimumab was found to slow radiologic progression of RA [46]. Adalimumab has also been studied in combination with methotrexate. A 24 week double-blind placebo-controlled trial compared adalimumab at 20 mg, 40 mg, or 80 mg biweekly with methotrexate, versus methotrexate alone [47]. The patients had relatively long-standing RA (mean 12.3 years), had been on a mean of 3 previous DMARDs, and continued to receive methotrexate (mean baseline dose 16.8 mg/wk). The results showed significantly greater efficacy for the adalimumab plus methotrexate groups than the placebo plus methotrexate group. There was significant improvement in ACR 20, ACR 50, swollen joint count, total joint count, and HAQ scores for all three adalimumab groups. For the ACR 20, the placebo group had a 14.5% response rate, while the adalimumab groups were at 47.8% (20 mg), 65.7% (40 mg), and 65.8 % (80 mg). Except for an increase in injection site reactions, the adalimumab groups had similar side effects to the methotrexate plus placebo group. From these initial studies, it appears that adalimumab is as effective in treating RA as infliximab, although head-to-head comparisons have not been performed. The mechanisms of action for the two drugs are also similar, with reduction in TNFα and IL-1β reported for adalimumab [48]. Reported adverse events have been identical in placebo groups compared to adalimumab groups, with the exception of injection site reactions. The safety of adalimumab has not been fully explored, with no available data on serious infections (except for one reported case of TB), malignancies, or development of anti-adalimumab antibodies.

A humanized monoclonal anti-TNFα antibody, CDP571, has been evaluated in the treatment of Crohn's disease. The drug was found to be effective for treating active Crohn's disease and possibly effective in closing fistulas and maintaining remission of disease [20, 21]. Side effects have included anti-idiotype antibodies, infusion reactions, and formation of autoantibodies. In RA, the drug was effective at a dose of 10 mg/kg but not at 1 mg/kg [49]. This meant that CDP571 was less effective in treating RA than infliximab at equivalent doses, and further trials have not been pursued for this indication.

A further anti-TNFα antibody evaluated in a clinical trial on RA patients is the PEGylated humanized anti-TNFα fragment CDP870 [50]. Modification of the antibody by PEGylation has increased its half-life to approximately 14 days. In an initial study, the drug or placebo was given as a single i.v. infusion in a double-blind, ascending dosage trial to 36 long-term (13 years of RA), refractory (mean of 5 prior DMARDs) patients. The ACR 20 at 8 weeks was 16.7% (placebo), 25% (CDP870 1mg/kg), 75% (CDP870 5mg/kg) and 75% (CDP870 20 mg/kg; $p = 0.03$). No excess of adverse events was found.

PEGylation has also been applied for extending the half-life of recombinant dimers of soluble TNFα type I receptors,

made in E. coli. In phase I/II trials in RA patients, this product was found to elicit a significant antibody response that adversely affected the half-life and clearance of the recombinant proteins [51]. These findings led to the conclusion that these receptor dimers were not a viable treatment. Instead, there has been development of PEGylated monomers of TNFα type I receptors. In a phase I study, the safety, pharmacokinetics, and clinical effectiveness have been investigated in RA patients [52].

Lenercept is a further TNFα type I receptor-human IgG1 Fc fusion protein developed for study in treating sepsis or RA. A 12 week dose-finding placebo-controlled, double-blind randomized study was performed in 247 patients to compare lenercept alone to methotrexate alone or to the combination of the two [53]. The ACR20 results were equivalent in all three groups, ranging from 53% (lenercept), 50% (methotrexate), to 64% (both drugs). Because the efficacy was equivalent but not superior to that of methotrexate, further trials of lenercept are not planned.

COMBINATION THERAPY WITH TNFα ANTAGONISTS

With inversion of the therapeutic pyramid in the treatment of RA, there has been increasing use of more aggressive, multiple drug therapy. This includes the triad of methotrexate, hydroxychloroquine, and sulfasalazine that has superior efficacy to one or two of the drugs alone [54]. Less experience exists in combinations incorporating TNFα blockers. Infliximab is given with low doses of methotrexate (7.5 mg p.o. q week) in order to prevent loss of efficacy from anti-infliximab antibody formation and not for added direct therapeutic benefit. However, clinical trials indicate at most a 68% ACR 20 response to infliximab, necessitating other approaches in the other 32% of patients. Whether pushing the methotrexate dose to high therapeutic levels (20 mg/week) in combination with infliximab provides a higher rate of response is still under investigation. Four studies have demonstrated the benefits of combining methotrexate and TNFα blockade (reviewed in [55]).

Combinations of biologicals are a logical progression from the combination therapy already in use for multiple inflammatory diseases with unknown etiologies. The potential benefits are additive or synergistic efficacy, fewer side effects from each agent, lower doses and therefore lower cost, and greater immunomodulation by attacking the inappropriate immune response at different sites [56]. Very few combinations of biologicals have been used in humans. The potential combinations include TNFα blockers with inhibitors of other cytokines or chemokines such as IL-1, IL-2, IL-12, and IL-18, with IL-4, with anti-T cell antibodies such as anti-CD4, with inhibitors of T cell costimulation (CD154, CD28), with inhibitors of adhesion molecules, or with angiogenesis inhibitors. The combination of two biologicals has been investigated in a small trial of TNFα blockade along with anakinra (IL-1 receptor antagonist) [57]. Clinical efficacy (improved tender and swollen joint counts, HAQ score, CRP, and ESR) was seen in a preliminary trial yet of the 58 patients studied, 4 serious infections were reported, including 2 pneumonias and 2 cases of cellulitis. Whether such concerns will continue to be seen in the follow-up of larger groups of patients has yet to be determined.

NEW INDICATIONS FOR TNFα INHIBITION

With the success of TNFα inhibitors in treating rheumatoid arthritis, Crohn's disease, and seronegative spondyloarthropathies, consideration has been given to testing TNFα blockade in a variety of inflammatory and autoimmune conditions. With reassurance about the relative safety of infliximab and etanercept, the main exceptions to further study are most infectious conditions, active malignancies (though the risks are mainly theoretical concerning reduced immune surveillance in the presence of TNFα blockade), systemic lupus erythematosus (because of the reports of anti-ds DNA antibodies and rare drug-induced SLE), and demyelinating conditions.

Both etanercept and infliximab have been studied in advanced congestive heart failure (CHF), a state with elevated TNFα levels [58]. For etanercept, the Recover and Renaissance studies involved 2048 total patients and showed no change in mortality or hospitalization rate for CHF over 96 weeks. For infliximab, a phase II study with 150 class III or IV CHF patients was terminated early because of an apparent increase in hospitalization rates and death in the treated group (death in 7 infliximab patients, 0 placebo patients). Whether CHF patients with less advanced disease, or heart transplant patients, who have especially high levels of TNFα, might benefit from the therapy has not been determined.

The need for new therapies to treat inflammation that remains unresponsive to multiple anti-inflammatory, immunosuppressive, and cytotoxic treatments has driven the use of TNFa blockade in a large number of different diseases. A partial listing of potential disease targets for TNFα inhibition is presented in (Table 2). Most of the applications listed have been derived from case reports, with controlled trials needed to ultimately characterize clinical effectiveness.

LIMITATIONS OF TNFα-BLOCKING BIOLOGICALS

Biologicals are proteins and can therefore be antigenic. This applies most directly to mouse antibodies or mouse-human chimeric antibodies (e.g. infliximab) where human anti-murine responses potentially limit the long-term efficacy of the biologic compound. Advanced methods of antibody generation, including CDR grafting to create humanized antibodies and phage display to produce entirely human antibodies have reduced antigenicity but cannot eliminate the possibility of anti-idiotypic antibodies, which can form regardless of species. De-aggregation of antibody preparations helps to reduce their immunogenicity and represents a technical step to decrease injection reactions and undesired immune responses. Overall, antibody responses must be considered a potential complication for all biologic compounds.

Table 2. Potential Disease Targets for TNFα Blockade

Rheumatologic	Cardiac
Rheumatoid arthritis	CHF
Spondyloarthropathy	Myocarditis
Ankylosing spondylitis	Cardiac hypertrophy after heart transplant
Psoriatic arthritis	
Sjogren's Syndrome	Heme/Onc
Myositis	Graft vs. host disease
Juvenile chronic arthritis	Myelodysplastic syndromes
Adult Still's disease	Langerhans' cell histiocytosis
Vasculitis	
Wegener's granulomatosis	
Behcet's syndrome	Skin
Relapsing polychondritis	Psoriasis
TNF-receptor-associated periodic syndrome	Pyoderma gangrenosum
	Sweet's syndrome
Eye diseases	Hidradenitis suppurativa
Uveitis	Cutaneous lupus
Scleritis	
Keratitis	Infectious
Retinal vasculitis	Hepatitis C, chronic
Corneal allograft rejection	Sepsis
Gastrointestinal	
Crohn's disease	
Ulcerative colitis	
Celiac disease	
Pulmonary	
Sarcoidosis	
Interstitial Lung Diseases	

Infliximab is a mouse-human chimeric antibody and leads to generation of human antichimeric antibodies (HACA) in 53% of patients receiving the low dose of 1 mg/kg [59]. However, at higher doses the frequency of HACAs actually decreases, apparently as a result of high zone tolerance, a well-studied immunologic phenomenon of decreased responsiveness to a large antigen dose [60]. In this way, HACAs appear in 21 % of patients receiving a 3 mg/kg dose of infliximab, and in 7% receiving 10 mg/kg. The problem has been further mitigated by the observation that concomitant administration of low-dose methotrexate (7.5 mg p.o. q week) further decreases the HACA response to 15% (1 mg/kg infliximab), 7% (3 mg/kg), and 0% (10 mg/kg). Etanercept is also immunogenic, with antibodies to the compound found in 16% of treated patients [61], but without apparent effect on long-term effectiveness of the treatment. In addition, antibodies against one biologic compound such as infliximab do not cross-react with multiple other biologics (e.g. etanercept, rituximab (anti-CD20), abciximab (anti-GPIIb/IIIa)) and therefore do not prevent the sequential or concomitant use of several biologic compounds in the same patient [62].

Also induced in up to 15% of patients receiving anti-TNFα antibody or fusion proteins is anti-double stranded DNA antibody. This is in turn associated with the development of drug-induced lupus, but in only 0.2% of patients treated with infliximab, for example [63]. Therefore,

most patients with the antibodies remained asymptomatic, and those with drug-induced lupus have generally had resolution of their findings with discontinuation of the biologic. The mechanism for generation of anti-ds DNA antibodies in this situation is not well-understood. However, two mouse models have shown an association of SLE with low levels of TNFα. The (NZB x NZW)F1 mice have genetically-determined low production of TNFα and their lupus nephritis and decreased survival can be treated with low doses of TNFα supplementation [64]. Furthermore, TNFα-deficient mice develop anti-ds DNA antibodies and are at risk of autoimmunity [65]. The proposed mechanism for these observations argues that TNFα is critical for cytotoxic T lymphocyte induction, which in turn suppresses autoimmunity by killing autoreactive B cells [66]. Therefore, the reduced level of TNFα is part of the background that predisposes susceptible individuals to developing anti-ds DNA antibodies, and rarely, a drug-induced lupus syndrome. It is still under study whether the potential for induction of anti-ds antibodies is a contraindication for treating the inflammation of SLE with anti-TNFα therapy.

The occurrence of demyelination during anti-TNFα therapy represents a further potential complication of this treatment. In a review of information in the FDA Adverse Event Reporting System, 19 treated patients with inflammatory arthritides experienced similar neurologic findings that included optic neuritis, central nervous system demyelination, and frank multiple sclerosis [67]. Seventeen of the 19 had received etanercept, two infliximab. The neurologic events were temporally related to the anti-TNFα therapy, and occurred on average 5 months into the therapy. All patients had partial or complete resolution off the therapy, and symptoms recurred in the one patient rechallenged with etanercept. The data reinforce the earlier findings that lenercept, a soluble dimeric p55 TNFR fusion protein, was associated with increased multiple sclerosis exacerbations in a double-blind, placebo-controlled study [68], and that two multiple sclerosis patients treated with infliximab showed more numerous MRI lesions and higher CSF lymphocyte counts after the infusions [69]. Animal models of multiple sclerosis indicate that the role of TNFα is particularly complex in this disease. While anti-TNFα therapy can prevent disease onset or improve established disease in mice, the total absence of TNFα in knockout mice actually leads to particularly severe demyelination that is improved by adding back low doses of TNFα [70, 71]. While statistics are still being compiled about the relative frequency of demyelination during anti-TNFα therapy versus the background frequency of multiple sclerosis in the population of patients with chronic inflammatory conditions, these drugs are currently avoided in patients with possible demyelination.

INFECTION AND ANTI-TNFα THERAPY

By reducing the activity of TNFα and the downstream cascade of proinflammatory cytokines such as IL-1 and IL-6, anti-TNFα biologics have the potential for reducing immune responses to infectious diseases. In clinical practice and post-marketing surveillance, no increased incidence of common bacterial and viral infections has been noted with either etanercept or infliximab. However, there has been an increased incidence of M. tuberculosis infection in patients taking infliximab.

There were 147,000 patients worldwide exposed to infliximab by March, 2001, and 70 cases of TB for a rate of 48 cases/100,000 patient-years. By comparison, the historic rate of TB in the US population or the US population of RA patients is 8.2 and 6.0/100,000 patient-years, respectively. 82% of all patients receiving infliximab resided in the United States, and 63% of that group had received the drug for Crohn's disease. However, 47 of the TB cases were in RA patients and only 18 in Crohn's patients. It must be remembered that RA patients have been treated with multiple infusions beginning with the earliest trials, while many Crohn's patients initially received only single or sporadic doses of infliximab. It was striking that the TB seen in infliximab patients was frequently a disseminated (25%) or extrapulmonary (56%) infection occurring mostly within the first 14 weeks of infliximab therapy, as would be found in reactivated TB [6]. Although 82 % of infliximab users were in the United States, only 17 of 70 (24%) of TB cases occurred in this country. Nevertheless, 64 of the 70 TB cases occurred in countries with low TB incidence, and even among the five US immigrants developing TB on infliximab, all had been in the US over 10 years.

The mechanism for this excess incidence of TB has not been elucidated. One proposal has been that infliximab is a complement-fixing antibody that binds to TNFα, even on cell surfaces, irreversibly and can cause lysis of the TNFα-bearing cell. If this cell is a macrophage harboring latent TB, the bacilli might be released and reactivated after infliximab kills the macrophage. Etanercept, which is a soluble receptor and does not fix complement or bind irreversibly to its target, is not associated with an excess incidence of TB (8 United States TB cases for a rate of 7.3/100,000). As more experience is being gathered, the clinical recommendation exists to test all infliximab candidates for TB by a tuberculin skin test, and to treat any positives with anti-tuberculous regimens before beginning infliximab therapy.

SMALL MOLECULE INHIBITORS OF TNFα

The necessity for injection or i.v. infusion of the commercially available TNFα blockers is a major hurdle for patient acceptance and increases the cost of production, storage, and delivery of these drugs. In addition, the clinical effectiveness of available products is lacking in a substantial percentage of patients, pointing out the need for new therapies. Therefore, there is considerable interest in small molecule inhibitors of TNFα and of the related signal transduction pathways that contribute to inflammation.

Beginning outside the cell, one approach is to target the TNFα trimer and prevent its interaction with TNFα receptors. This is the mechanism of action for the structural analogues of suramin, a symmetrical polysulfonated urea derivative that promotes the dissociation of human TNFα trimer into biologically inactive subunits [72]. Molecular

modeling studies are revealing how these molecules interact with TNFα and prevent binding to the p55 TNFα receptor.

At the cell surface, potential strategies are physical blockade or disruption of the TNFα receptors, either by binding of foreign molecules to the receptors or by disruption of receptor synthesis. The latter approach has been utilized by the study of modified antisense oligonucleotides to TNFα RI that inhibit the receptor and therefore reduce signaling mediated by TNFα [73]. The compounds were partial phosphorothioate oligodeoxyribonucleotides containing C-5 propynyl or hexynyl derivatives of 2'-deoxyuridine, and the most active ones targeted the 3'-polyadenylation signal on the TNFαRI mRNA. A second approach has been the use of morpholino antisense oligomers to TNFα. When delivered intranasally 12 hours prior to lipopolysaccharide (LPS) challenge, the oligomers were able to specifically and consistently inhibit TNFα production in mice [74]. For use *in vivo*, delivery and stability of these compounds are hurdles that need to be overcome. One new experimental method of delivering antisense phosphorothioate oligonucleotides has been encapsulation in pH-sensitive liposomes. These liposomes are stable for 4 weeks at pH 7.4 but release their contents below pH 6, such as is found in the early endosomes of macrophages. Rats treated with liposome-encapsulated antisense TNFα oligonucleotides showed up to 70% reduction in plasma TNFα production after LPS challenge compared to control animals [75].

Still at the cell surface, blockade of TACE (TNF-Alpha Converting Enzyme), the enzyme that cleaves the membrane-integral proTNFα to its 17 kDa soluble form, represents a potential target for inhibitors. TACE is a member of the adamalysin (or ADAM) family of matrix metalloproteinases (MMPs) with similarity in structure to other MMPs at its active site [76]. Other MMPs besides TACE have the capability of processing pro-TNF [77], but the primacy of TACE in the release of TNFα was suggested by studies of mouse cells deficient in TACE, showing markedly reduced levels of TNFα release [78]. A number of broad-spectrum metalloproteinase inhibitors have been described in the literature (marimastat (British Biotech), TAPI (Immunex), GI129471X and GI5402 (Glaxo), SE205 and XS309 (DuPont), CGS 27023A (Ciba), and several thiol inhibitors (Chiroscience)) whose mechanism of action is usually to inactivate the MMPs by binding to the zinc moiety [79]. The potential *in vivo* effects of blocking the shedding of multiple other cell-surface proteins besides TNFα (such as TGFα, L-selectin, amyloid precursor protein, p55 and p75 TNFα receptors, type II IL-1 receptor) has not been studied in detail but points to the potential need for more specific inhibitors of TACE (reviewed in [80]). The inhibitor GI5402 has been studied in humans to specifically define the effect on TNFα after *in vivo* LPS administration [81]. The drug strongly reduced the release of circulating TNFα by LPS but had no effect on monocyte-bound TNFα. There was no evidence for a drug-induced increase in cell-associated TNFα, an effect seen *in vitro* in TACE-deficient cells and concerning for production of a proinflammatory state [82]. The fact that soluble TNFα receptors as well as TNFα itself are released from the cell surface by TACE points out the potential for TACE blockade to lead to reduced sequestration of free TNFα, resulting in net pro-inflammatory effects. The usefulness of TACE as a target in the treatment of inflammatory arthritis has been further brought into question by the demonstration of severe arthritis in transgenic mice with only the membrane and not the soluble form of TNFα [83]. The two commercially available products for blockade of TNFα in humans, infliximab and etanercept, block the activity of both forms of TNFα.

INTERFERENCE WITH INTRACELLULAR SIGNALING

A vast number of potential targets exist when considering the steps that follow TNFα receptor engagement and end in the transcription of multiple proinflammatory genes. Interaction of TNFR1 with TNFα leads to the assembly of a complex including TRADD (TNF Receptor-Associated Death Domain protein), FADD (Fas-Associated Death Domain protein), caspace 8/FLICE, RIP 1 and RIP2 (Receptor Interacting Protein), and TRAF-2 (TNF Receptor-Associated Factor). On the other hand, TNFR2 is phosphorylated by casein kinase-I, interacts with TRAF-1, TRAF-2, and may function in part by passing its TNFα ligand to TNFRI. Depending on the balance of stimuli, TNFRI can lead to apoptosis via the caspases pathway, or to cellular activation downstream of TRADD and TRAF2 by activating MAP kinase and NF-κB pathways. A challenge in designing small molecule inhibitors will be to control both the apoptotic and activating pathways at once rather than dramatically shifting the balance with unforeseen side effects.

More distally in the signal transduction pathways, the NF-κB and MAP kinase cascades represent further potential targets for controlling inflammation. The activation of the NF-κB pathway after TNFRI engagement provides resistance to apoptosis if NF-κB-mediated transcription is turned on. In part, this effect stems from the induction of anti-apoptotic genes by NF-κB, including IEX-1L and A20 [84, 85]. A further potential drug target is the natural inhibitor IκB, that complexes with NF-κB to sequester it in the cytoplasm, and which is degraded to allow NF-κB to translocate to the nucleus for gene activation. Animals with expression in the liver of a transgenic degradation-resistant IκB repressor had an increased susceptibility to infection, indicating that short-term or adjuvant use of NF-κB might be necessary rather than wholesale blockade of this multifunctional pathway [86, 87]. A further approach has been the development of pyrimidine-containing compounds that inhibit NF-κB as well as transcription factor AP-1 gene expression, providing simultaneous inhibition of two proinflammatory pathways [88]. Clinical trials are not yet available.

The MAP kinases ERK, JNK, and p38 all lie downstream of TRAF2 in the signaling pathway from the TNFα receptor I. In turn, signaling through these MAP kinases leads to activation of transcription factor families such as the AP-1 and CREB/ATF proteins, which are important positive regulators of multiple proinflammatory genes. Therefore, controlling the activity of MAP kinases provides a possible avenue for regulating TNFα-induced inflammation.

The ERK pathway inhibitor PD 098059 has been studied *in vitro* and shown to reduce IL-6 gene expression after stimulation of mouse fibrosarcoma L929sA cells with TNFα [89]. *In vivo* trials have not been reported for ERK pathway inhibitors. A specific inhibitor of JNK, SP600125, has recently been investigated in rheumatoid arthritis since high levels of activated JNK are found in RA fibroblast-like synoviocytes [90]. The JNK inhibitor completely blocked IL-1-induced transcription factor AP-1 binding and collagenase mRNA accumulation, while blockade of ERK and p38 had minimal effects. In rat adjuvant arthritis, SP600125 modestly decreased paw swelling but caused nearly complete inhibition of radiographic damage. This would argue that blockade of the JNK pathway downmodulates mainly IL-1 effects, which are of prime importance in mediating the bony damage of RA.

The blockade of p38 has been pursued by multiple pharmaceutical companies. P38 represents a mediator of inflammatory cytokine signaling that also lies upstream of TNFα and IL-1 production, making it a potential target for interrupting an inflammatory circuit. The lead compound developed by Vertex Pharmaceuticals was VX-745, which advanced to a 12-week, randomized, placebo-controlled phase II trial in rheumatoid arthritis. The results using the lower of two planned doses showed promising reductions in the ACR 20 as a measure of inflammation, but further development was halted due to concerns of neurologic side effects from the drug in animal models. A trial of VX-745 in myelodysplastic syndrome was also halted. The dose used in animal testing was 10 times that used in the clinical trials, and no side CNS side effects were reported in humans. Vertex is now developing second generation p38 inhibitors that do not cross the blood-brain barrier, VX-702 and VX-850, currently nearing Phase I trials in humans. Aventis Pharma has reported *in vitro* and *in vivo* studies of a novel p38 inhibitor, RPR132331. In rat streptococcal cell wall arthritis, the drug reduced the incidence and progression of disease, whether given prophylactically or therapeutically [91]. SmithKline Beecham has developed a second generation p38 inhibitor, SB 239064, that shows improved kinase selectivity and increased *in vivo* activity compared to the first generation compound [92]. In animal testing, SB29064 inhibited LPS-induced TNFα production and reduced rat adjuvant-induced arthritis by 51%. In addition, the compound reduced infarct volume by 48% and neurologic deficits by 42% when given before a moderate stroke in rats.

For the treatment of Crohn's disease, the JNK/p38 inhibitor CNI-1493 has been studied in initial trials. CNI-1493 is a guanylhydrazone compound that was used at 8 or 25 mg/m^2 daily for 12 days in 12 patients. The results demonstrated that colonic biopsies, which had activated JNK and p38 MAP kinases initially, had inhibition of phosphorylation of both enzymes and diminished TNFα production after the treatment. Clinically, there was a significant decrease in disease activity measures in 67% of patients at week 4, including rapid endoscopic ulcer healing, endoscopic improvement in 11 or 12 patients, healing of fistulae in 4 of 5 patients, and reduction of steroid dosage in 89%. A response was seen in 3 of 6 patients who had been infliximab failures [93]. Although this trial was small and uncontrolled, MAP kinase inhibition presents a new therapeutic strategy in Crohn's disease that will be investigated further.

The synthesis of TNFα itself is a target for thalidomide. Although the exact mechanism of action is still unclear, thalidomide has been reported to selectively increase the rate of TNFα mRNA degradation [94] and also has modest antiangiogenic effects. Clinically, thalidomide is the drug of choice for erythema nodosum leprosum, and has also been used in multiple sclerosis, Crohn's disease, and the chronic diarrhea and wasting associated with HIV infection. There are also promising small trials to evaluate thalidomide in the treatment of rheumatoid arthritis, ankylosing spondylitis, and chronic graft-versus-host disease (reviewed in [95] and [96]). The use of thalidomide is limited by its potential for severe teratogenic and neurotoxic side effects. Therefore, thalidomide derivatives that may have less toxicity and enhanced anti-TNFα activity are under development [97].

A further action of thalidomide is to block phosphodiesterase IV, the main cellular activity that degrades cyclic AMP (cAMP) [98]. The resulting elevated intracellular levels of cAMP in leukocytes have anti-inflammatory effects, with decreased macrophage TNFα production and secretion, decreased IL-1β production, and inhibited T cell activation [99, 100]. One phosphodiesterase IV inhibitor, rolipram, has been used in the collagen-induced arthritis model in mice and found to be effective in reducing joint inflammation whether given before or after induction of arthritis [101-103]. Further development of phosphodiesterase IV inhibitors may represent a way of exploiting some of thalidomide's beneficial properties while avoiding its serious toxicities.

FUTURE ISSUES

The development of agents to block TNFα in the treatment of inflammatory diseases is still in its early stages. As more experience about the currently available agents is gained and as new generations of TNFα inhibitors become available, the disease indications and the number of treatment options will increase. Several issues are already emerging for the future:

1. Combinations of drugs. It is clear from studies in rheumatoid arthritis, Crohn's disease, and spondyloarthropathies that some patients do not respond to standard doses of anti-TNFα agents. The ongoing use of these drugs in partial responders, including the use of escalating doses, as well as the initiation of a second TNFα blocker after failing a previous one remain insufficiently studied. Therefore, further investigation will be directed at combinations of agents including TNFα blockers with methotrexate, leflunomide, cyclosporin-A, biologics targeting other cytokines, and other standard therapies already in use. To date, clinical trials of TNFα blockers have not included study arms to test the biologic agent alone versus a combination of TNFα blockers with older agents, making it impossible to examine an additive or synergistic clinical effect of

the TNFα blockers. Once trials of p38, JNK, and NF-κB inhibitors progress sufficiently, they are also likely to be added to the available therapies.

2. Discontinuation of TNFα antagonists. It is rare to achieve a cure or permanent remission in chronic inflammatory conditions such as rheumatoid arthritis. Therefore, standard therapy has included long-term administration of disease-modifying agents and only cautious taper and discontinuation of therapy in a select few patients with no evidence of disease activity over months to years of follow-up. Given the costs and inconvenience of taking injected TNFα blockers, there will be an incentive to reduce the dose, increase the interval of administration, substitute a different anti-inflammatory drug, or discontinue treatment entirely. At this time, there is no experience to address the consequences of these courses of action.

3. Identification of patients early in their disease. The etiology of most chronic inflammatory disease is unknown, but experience with infections such as Lyme disease demonstrates that chronic inflammation can persist even after the causative agent has been eradicated. Therefore, the goal of identifying the original cause of chronic inflammatory conditions will be of interest but may not lead to effective therapy for those with established disease. The goal of therapy in this circumstance is to interrupt the self-sustaining inflammatory process and reset the immune response. Beginning treatment early in the disease course, and making progress towards identifying those patients destined to have the most severe disease, remain goals for minimizing destructive consequences. However, clinical trials are needed to prove that early, aggressive therapy with TNFα blockade is actually superior to currently-available therapy.

4. Safety. Experience is still accumulating about the safety of infliximab and etanercept, with no long-term results available for other agents. By disrupting the signaling of the TNFα pathway, these agents may theoretically impair clearance of infections and predispose to autoantibody or cross-reacting antibody formation as already described. Other long-term effects on immune-surveillance, including the risk of malignancy, are unproven at this time. It must be remembered that patients with chronic immune stimulation from rheumatoid arthritis are already at increased risk of malignancy. What is unknown, however, is whether tight control of the inflammatory process could actually decrease the risk of cancer by lessening the abnormally active immune response.

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